SUPPORTING INFORMATION

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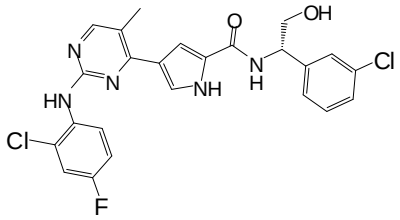
Contents of Supporting Information:

1. Description of biochemical and cellular assays.

ERK Inhibition Assay: Compounds were assayed for the inhibition of ERK2 by a spectophotometric coupled-enzyme assay as described in Fox et al. (Fox, T., Coll, J.T., Xie, X., et al. *Protein Science* **1998**, *7*, 2249-2255). In this assay, a fixed concentration of activated ERK2 (10 nM) was incubated with various concentrations of the compounds in DMSO (2.5%) for 10 min. at 30 °C in 0.1 M HEPES buffer, pH = 7.5, containing 10 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate, 200 μM NADH, 150 μg/mL pyruvate kinase, 50 μg/mL lactate dehydrogenase and 200 μM erktide peptide. The reaction was initiated by the addition of 65 μM ATP. The rate of decrease of absorbance at 340 nM was monitored. The IC_{50} was evaluated from the data as a function of inhibitor concentration.

Kinase Counterscreening: % Inhibition values below were measured at Upstate/Millipore (2 μM) and Nanosyn (1 μM).

Table S1. Kinase counterscreening data for **11e**.



Assay	K_I, μM
ERK2	< 0.002
GSK3	0.40
AURA	0.54
CDK2	0.85
FLT3	1.4
ROCK1	1.4
JNK3	1.4

TAK1	3
PKA	> 4
SRC	> 4
JAK2	> 4
JAK3	> 4
SYK	> 4
MET	> 4
IRAK4	> 4
AKT3	> 4
KDR	> 4
MEK1	> 4
P38α	> 4
COT	> 4
ITK	> 4
MEKK3	> 4
PLK1	> 4
ZAP70	> 4
IKKi	> 5
NIK	> 5
CIT	> 10
FAK	> 10
IRAK1	> 10
MLK2	> 10

NAK	> 10
PDK1	> 10
SGK1	> 10

Additional counterscreening data (% INH at fixed concentration)

ABL1 (%INH @ 1 uM): 3
AKT1 (%INH @ 1 uM): 0
AKT2 (%INH @ 1 uM): 0
AKT3 (%INH @ 1 uM): 0
AMPK (%INH @ 2 uM): 5
BLK (%INH @ 2 uM): 7
BMX (%INH @ 1 uM): 3
BRSK1 (%INH @ 1 uM): 0
BRSK2 (%INH @ 1 uM): 2
BTK (%INH @ 1 uM): 5
CAMKII (%INH @ 2 uM): 1
CAMKIV (%INH @ 2 uM): 0
CDK1 (%INH @ 1 uM): 7
CDK5 (%INH @ 1 uM): 24
CHK1 (%INH @ 2 uM): 40
CK1 (%INH @ 2 uM): 25
CK2 (%INH @ 2 uM): 0
CLK3 (%INH @ 1 uM): 9
CSK (%INH @ 2 uM): 0
CSK (%INH @ 1 uM): 3
DAPK1 (%INH @ 1 uM): 2
DCAMKL2 (%INH @ 1 uM): 1
DYRK1A (%INH @ 1 uM): 3
DYRK2 (%INH @ 1 uM): 3
EGFR (%INH @ 1 uM): 6
EPH-B2 (%INH @ 1 uM): 0
EPH-B4 (%INH @ 1 uM): 0
FER (%INH @ 1 uM): 3
FGFR1 (%INH @ 1 uM): 3
FGFR2 (%INH @ 1 uM): 12
FGFR3 (%INH @ 1 uM): 8
FGFR4 (%INH @ 1 uM): 0.3
FLT-1 (%INH @ 1 uM): 0
FLT-4 (%INH @ 1 uM): 8
FMS (%INH @ 1 uM): 6
FYN (%INH @ 2 uM): 11

HCK (%INH @ 1 uM): 3
IGF1R (%INH @ 1 uM): 2
IKKa (%INH @ 2 uM): 0
IKKb (%INH @ 2 uM): 8
INSR (%INH @ 1 uM): 4
IRR (%INH @ 1 uM): 0
JNK1 (%INH @ 2 uM): 34
JNK2 (%INH @ 2 uM): 43
KIT (%INH @ 1 uM): 8
LCK (%INH @ 2 uM): 3
LYN (%INH @ 2 uM): 7
MAP4K4 (%INH @ 1 uM): 18
MAPKAPK-2 (%INH @ 1 uM): 0
MAPKAPK-3 (%INH @ 1 uM): 5
MARK1 (%INH @ 1 uM): 12
MER (%INH @ 1 uM): 9
MINK (%INH @ 1 uM): 18
MKK4 (%INH @ 2 uM): 69
MKK6 (%INH @ 2 uM): 9
MKK7 (%INH @ 2 uM): 6
MSK1 (%INH @ 1 uM): 7
MSK2 (%INH @ 1 uM): 6
MST1 (%INH @ 1 uM): 4
NEK1 (%INH @ 1 uM): 6
NEK2 (%INH @ 1 uM): 3
P38β (%INH @ 2 uM): 25
P38δ (%INH @ 2 uM): 54
P38γ (%INH @ 2 uM): 56
P70S6K (%INH @ 2 uM): 18
PAK1 (%INH @ 1 uM): 3
PAK2 (%INH @ 1 uM): 0
PAK3 (%INH @ 1 uM): 3
PAR-1Bα (%INH @ 1 uM): 5
PASK (%INH @ 1 uM): 19
PDGFRα (%INH @ 2 uM): 3
PDK1 (%INH @ 2 uM): 10
PHKγ2 (%INH @ 1 uM): 4
PIM1 (%INH @ 1 uM): 0
PIM2 (%INH @ 1 uM): 3
PKCβ1 (%INH @ 1 uM): 4
PKCη (%INH @ 1 uM): 2
PKCα (%INH @ 2 uM): 12
PKCβ2 (%INH @ 2 uM): 4
PKCγ (%INH @ 2 uM): 11
PKCθ (%INH @ 2 uM): 17

PRK2 (%INH @ 2 uM): 14
PRKD1 (%INH @ 1 uM): 43
PRKD2 (%INH @ 1 uM): 50
PRKD3 (%INH @ 1 uM): 21
PRKX (%INH @ 1 uM): 3
PYK2 (%INH @ 1 uM): 6
RAFc (%INH @ 2 uM): 3
RET (%INH @ 1 uM): 3
ROCK II (%INH @ 2 uM): 57
RON (%INH @ 1 uM): 1
ROS (%INH @ 1 uM): 3
RSK1 (%INH @ 1 uM): 4
RSK2 (%INH @ 1 uM): 6
RSK3 (%INH @ 1 uM): 7
RSK4 (%INH @ 1 uM): 11
SGK1 (%INH @ 1 uM): 15
SGK2 (%INH @ 1 uM): 41
SGK3 (%INH @ 1 uM): 2
TBK1 (%INH @ 1 uM): 3
TIE2 (%INH @ 1 uM): 0
TSSK2 (%INH @ 1 uM): 3
TYRO3 (%INH @ 1 uM): 0
YES (%INH @ 1 uM): 5

ERK Inhibition Cell Proliferation Assay:

The cell line, Colo205, was obtained from ATCC. Colo205 proliferation was measured by ^{3}H-thymidine incorporation. The cells were plated at a concentration of 10,000 cells/well in a 96-well plate using growth media, RPMI 1640 containing 10% FBS. Serially diluted compounds were added. The cells and compounds were incubated for 48 hours at 37°C incubator. After 48 hours 0.4 µCi of ^{3}H-thymidine (NEN, Cat. #NET-027) was added to each wells for 8 hours and returned to the 37°C incubator. The cells were harvested using a Tomtec 96-well cell harvester and the CPM was determined using the Wallac 1205 BETAPLATE liquid scintillation counter. The IC50 is the 50% inhibition of control (cells with vehicle).

2. PK data for 11e.

Mol.Wt. 499; logP = 5.1; logD = 4.3; solubility (pH 7.4) 10 µM; human plasma PB 99% at 1 µM.

Rat *iv* PK (3 mg/kg; DMI): Cl = 24 mL/min/kg; $t_{1/2}$ = 3.2 h.; Vss = 4.9 L/kg; AUC (0-8h) = 1.6 μg*h/mL; C_{4h} = 123 ng/mL; C_{8h} = 50 ng/mL.

Rat *po* PK (10 mg/kg; 0.5% MC, 1% SLS): F = 65%; $t_{1/2}$ = 3 h; C_{4h} = 329 ng/mL; C_{8h} = 267 ng/mL.

Mouse *iv* PK (5 mg/kg; DMI): Cl = 55 mL/min/kg; $t_{1/2}$ = 1.6 h.; Vss = 5.6 L/kg; AUC (0-8h) = 1.6 μg*h/mL; C_{4h} = 79 ng/mL; C_{8h} = 17 ng/mL.

Mouse *po* PK (33 mg/kg; 0.5% MC, 1% SLS): F = 67%; $t_{1/2}$ = 4.4 h; C_{4h} = 488 ng/mL; C_{8h} = 122 ng/mL.

3. Crystallographic Information.

Methods: Erk-2 protein was expressed, purified and crystallized as described in Fox et al. (Fox, T., Coll, J.T., Xie, X., et al. *Protein* Science **1998**, *7*, 2249-2255). Briefly, full-length ERK1 (Met1-Ser360) was expressed as a fusion with an N-terminal $(His)_6$ tag in *E. coli*. The protein was purified using a combination of metal-affinity chromatography (Talon resin) and anion-exchange chromatography (Q sepharose resin). Crystals were grown by vapor diffusion using a reservoir solution containing 100 mM MES buffer, pH 6.5, 26-28% PEG-MME 2000, 200 mM ammonium sulfate and 20mM 2-mercaptoethanol.

JNK-3 protein was expressed, purified and crystallized as described in Xie et al. (Xie, X., Gu, Y., Fox, T., et al. *Structure* **1998**, *15*, 983-991). Briefly, a truncated JNK-3 construct (Ser40-Glu402) was expressed in *E. coli*. The protein was purified using cation-exchange chromatography (SP Sepharose resin). Crystals were grown by vapor diffusion using a reservoir solution containing 20-24% PEG-MME 550, 10% ethylene glycol, 100 mM HEPES buffer, pH 7.5, and 20mM 2-mercaptoethanol.

X-ray data were collected on an Raxis IIC image plate and processed using DENZO/SCALEPACK. Model building and refinement were performed using Quanta and CNX respectively (Accelrys).

X-ray statistics:

protein	ERK2	ERK2	GSK3
compound	**9a**	**2**	**2**
Data collection			
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell parameters	44.1 x 70.3 x 119.6 Å	43.9 x 70.8 x 118.5 Å	82.75 x 86.1 x 178.29 Å
Resolution (Å)	20 – 2.5	20 - 2.2	37.3 – 2.3
Redundancy	3.4	3.4	6.3
Completeness (%)*	95.5 (92.0)	95.2 (75.4)	95.8 (78.9)
R_{merge}*	0.043 (0.29)	0.065 (0.28)	0.057 (0.26)
$\langle I/\sigma \rangle$*	12.9 (3.8)	19.5 (7.2)	22.3 (7.2)
Refinement			
Reflections used	12456	18070	51693
Test reflections	1012	1421	2635
R-factor	0.207	0.214	0.179
Free R-factor	0.248	0.257	0.222
RMS deviations			
Bond lengths (Å)	0.008	0.009	0.012
Bond angles (°)	1.05	1.16	1.22
Dihedral angles (°)	21.1	21.6	17.6Í

*Values for the highest resolution shell are shown in parentheses.

$R_{merge} = \sum_{hkl} \sum_i \left| I(hkl)_i - \langle I(hkl) \rangle \right| / \sum_{hkl} \sum_i \langle I(hkl)_i \rangle$ over i observations of reflection hkl.

R-factor = $\sum \left| |F_{obs}| - |F_{calc}| \right| / \sum |F_{obs}|$ where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. Free R-factor is calculated from a randomly chosen subset of reflections not used for refinement.